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[COMMUNICATION]

Induction of Male Sexual Behaviors by Administration of Testosterone Using Silastic Tubes in Castrated Male and Female Rats

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ABSTRACT—In order to determine a suitable length of Silastic tubes (3.18 o.d.×1.57 i.d. mm Dow-Corning) containing testosterone (T) for induction of normal level of male sexual activity, two 5 cm (T10 group) or two3 cm tubes (T6 group) were implanted subcutaneously in sexually inexperienced castrated males. T10 males showed stronger sexual activity than T6 males and intact control males. Sexual activity of T6 males was almost the same level as that intact males. Ovariectomized females with two 5 cm T-tubes (F10) showed only low level of mounting activity. These results indicate that implantation of two 3 cm Silastic tubes containing T is useful to induce normal level of male sexual activity in castrated male rats.

INTRODUCTION

Adult male rats show a series of male sexual behaviors-mount, intromission and ejaculation, when they find an estrous female [1, 2]. Castration depresses the display of these sexual behaviors and androgen replacement therapy restores these behaviors in males [3–5]. Antiandrogen also suppresses sexual behaviors of males [6]. These results indicates that male sexual behaviors dependent on the blood level of androgen. However, in order to induce the complete male copulatory behavior in castrated male rats, successive subcutaneous injections of androgen for a long period are necessary [7, 8]. Wada [9] reported that administration of androgen using Silastic tubes instead of injections was effective to induce male sexual calling in castrated male Japanese quails. In the present report, since Silastic tubes has been reported to be useful in maintaining a constant blood level of steroids [10, 11], two kinds of length of Silastic tubes containing testosterone (T) were implanted subcutaneously in castrated male rats, in order to restore normal level of behavioral activity.

MATERIALS AND METHODS

Sexually inexperienced Wistar males (210–250 g) and female rats (255–290 g) were maintained under controlled photoperiod (14:10 hr, light:dark) and temperature (24–25°C). Twenty-two males and 7 females were castrated under anesthesia. Seven males without castration served as intact controls. Three weeks after castration,all castrated animals received subcuatenous implantation of T(Sigma) using Silastic medical grade

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tubing (3.18 mm o.d.×1.57 mm i.d., Dow-Corning, Michigan) according to the technique of Legan *et al.* [10] and Nash *et al.* [11]. Silastic tubing was cut into 3 or 5 cm segments and one end was sealed with Silastic adhesive (Dow-Corning). After allowing to dry, each tube was loosely packed with T and sealed completely. The mean contents of T in 3 and 5 cm tubes were 20.6±0.5 mg and 43.9±1.0 mg, respectively. Before implantation, all tubes containing T were incubated overnight in saline solution. Eight castrated males were subcutaneously implanted with two 3 cm tubes containing T in the right and left back (T6). In other 8 castrated males, a pair of 5 cm tubes with T was implanted (T10). Six castrated males received vacant tubes (controls, T0). In addition, 7 ovariectomized rats were implanted with two 5 cm tubes containing T (FT10).

Behavioral tests were carried out on days 5, 10, 15 and 20 following T implantation. Each experimental rat was adapted in an observation cage (60×50×40 cm) for 3~4 min. Then, a receptive female pretreated with estrogen and progesterone prior to the test was placed with the experimental animal. The observation was continued for 30 min. The receptive female was replaced by another receptive female every 10 min in order to diminish the influence of affinity between the experimental animal and the female. The following standard measures were recorded; mount frequency (MF, number of mounts with thrust and with or without intromission from the start to the first ejaculation or during 30 min if no ejaculation); intromission frequency (IF, number of mounts with intromission); ejaculation frequency (EF, number of ejaculation during 30 min); mount, intromission and ejaculation latencies (ML, IL, EL, time from the introduction of the receptive female to the first occurrence of each behavioral pattern). MF and IF were indicated after converting to the frequency per 5 min. After the end of the test series, all tubes implanted in order to check the reduced amounts of T during implantation in each tube.

RESULTS AND DISCUSSION

All of 6 castrated males which received the vacant tubes (T0) showed no sexual behavior throughout. As shown in Figures 1 and 2 and Table 1, the incidence of mounts and intromissions in T0 and intact control males were low in the first test, being not significantly different from that of T0 group. No significant difference in the mean MF and IF was detected among T6, intact and T0

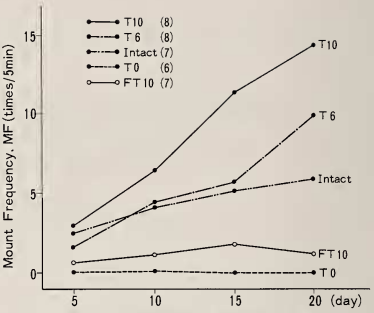

FIG. 1. Effect of subcutaneous implantation of Silastic tubes containing testosterone (T) on mean mount frequency (MF). Castrated male rats were implanted with two 3 cm (T6), two 5 cm (T10) or vacant (T0) tubes. Ovariectomized female rats were implanted with two 5 cm tubes (FT10).

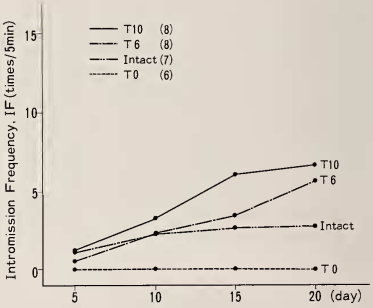

FIG. 2. Effect of implantation of T on mean intromission frequency (IF) in intact males, castrated males and females. T6, castrated males implanted with two 3 cm Silastic tubes containing T; T10, castrated males with two 5 cm tubes; T0, castrated males with vacant tubes.

Table 1. Incidence (I) and latency (L) of mount and intromission behaviors in each group

Group	DAY 5		DAY 10		DAY 15		DAY 20	
	I	L	I	L	I	L	I	L
MOUNTING								
T6	3/8	125	7/8	18	7/8	14	7/8	8
T10	6/8	311	8/8	14	8/8	5.5	8/8	6
Intact	4/7	70	5/7	11	6/7	9	6/7	46.5
Female	2/7	460.5	4/7	464	4/7	23	4/7	64
INTROMISSION								
T6	2/8	811	5/8	158	6/8	15	7/8	17
T10	5/8	502	7/8	79	8/8	12.5	8/8	18
Intact	4/7	109.5	5/7	60	6/7	22.5	6/7	491
Female	0/7	—	1/7	935	1/7	222	1/7	65

The data in the T0 group are not included in this table, because they did not show male sexual behaviors in 4 tests. L (see): median value.

Table 2. Incidence (IE), frequency (EF) and latency (EL) of ejaculation in each group

Group	DAY 10			DAY 15			DAY 20		
	IE	EF+SEM	EL	IE	EF+SEM	EL	IE	EF+SEM	EL
T6	1/8	0.1±0.1	1037	3/8	0.6±0.3	631	3/8	0.6±0.4	1389
T10	3/8	0.5±0.3	1487	6/8	1.0±0.3	1201.5	7/8	1.6±0.3	860
Intact	1/7	0.3±0.3	651	2/7	0.4±0.3	931	3/7	0.6±0.3	1297

The data in the T0 and female groups are not included in this table, because none of them show ejaculatory pattern throughout tests. EL (sec): median value.

groups. Only one intact males achieved to ejaculation in the first test (Table 1). In T10 group, the incidence ($p<0.05$, X^2-test) and frequency ($p<0.05$, t-test) of mounts (but not intromission) were significantly greater than those in the T0 group. In the second test (day 10), the mean MF and IF of T10, T6 and intact males were significantly larger than those of T0 males. The mean MF and IF of T10 males were still comparable to those of T6 and intact males. In the third test (day 15), however, T10 males became sexually more active, compared to T6 and intact males. The mean MF and IF of T10 males were 11.4±1.2 and 6.1±1.2, respectively, being significantly greater than those of T6 males (MF=5.7±1.9, IF=3.4±1.9) and intact males (MF=5.2±1.7, IF=2.6±1.1) ($p<0.025$). Ejaculation was observed in 6 of 8 T10, 3 of 8 T6 and 2 of intact males in the last test (day 20, see Table 2). The mean MF (14.5±1.4) and IF (6.7±0.9) in T10 males was significantly higher than those in the intact males (MF=5.9±1.8, IF=2.7±1.3) ($p<0.01$ and $p<0.05$, respectively). The ML and IL in T10 males were significanlly shorter than those of intact controls ($p<0.05$, U-test). In T6 males, the mean MF (9.9±2.4) and IF (5.8±1.9) were not significantly different from those in intact males. T10 males showed ejaculation more frequently than the males of other groups and the mean EF (1.6±0.4) during 30 min was significantly higher than those in T6 and intact males (0.6±0.4 and 0.6±0.3 respectively) ($p<0.05$, t-test). The mean amount of T decreased during 20 days implantation was 3.5±0.7 mg in cm Silastic tubes and 6.6±0.7 mg in 10 cm tubes, respectively.

Thus, the sexual activity in all groups except T0 increased gradually in 4 tests. Cumulative hor-

monal effects may be the most important factor for restoration of sexual behavioral activity in castrated male rats. However, the experience and habituation to testing as an ifluencing factor cannot be exculded, because an increase in behavioral activity was observed in intact males during the curse of the experiment.

The result that castrated males with two 5 cm tubes containing T (T10) showed significantly higher sexual activity than males with two 3 cm tubes (T6) in the present study indicates that male sexual activity is dependent on the length of Silastic tube containing T when implanted subcutaneously to castrated males. To induce sexual activity comparable to that of intact males, implantation of two 3 cm tubes filled with T appears to be enough. Since approximately 3.5 mg of T would be released from 3×2 cm tube for 20 days, T6 males may receive 175 μg of T daily for 20 days. In our previous study, daily injections of 500 μg testosterone propionate (TP) for 21 days were necessary to induce intact male levels of male sexual behaviors in castrated male rats [8]. In general, TP is thought to be more active than T [12] and the effect of the propionate derivative lests longer. Thus, the use of Silastic tubes for androgen administration seems to be quite favorable to induce male sexual behaviors in castrated males, because a smaller dose of T given by Silastic tubes is enough to induce normal levels of male sexual activity in castrated males. This may be because Silastic tubes can allow the continuous release of steroid contained [10].

In the females with two 5 cm tubes containing (F10), none showed ejaculatory pattern and only one female displayed intromission pattern throughout the test series. Mounting behavior was observed in 4 of 7 females and the mean MF was 0.7 ± 0.5, 1.2 ± 0.5, 1.8 ± 0.9 and 1.2 ± 0.6 in 4 tests, respectively. This result confirmed the previous reports that masculine sexual behaviors are very rare in ovariectomized female rats even when treated with high doses of androgn [8, 13].

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